

## Iron Deficiency Anemia Is Highly Prevalent among Human Immunodeficiency Virus–Infected and Uninfected Infants in Uganda<sup>1</sup>

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**ABSTRACT** Although anemia is a common finding among human immunodeficiency (HIV)-infected infants in sub-Saharan Africa, the factors contributing to the pathogenesis of anemia have not been well characterized. We sought to characterize the relative contribution of iron deficiency and chronic disease to the anemia among infants. Hemoglobin, ferritin, erythropoietin, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), neopterin, CD4<sup>+</sup> lymphocyte count and plasma HIV load were measured in 165 HIV-infected and 39 uninfected 9-mo-old infants seen in an outpatient pediatric clinic in Kampala, Uganda. Among HIV-infected and uninfected infants, the prevalence of anemia (hemoglobin < 110 g/L) was 90.9 and 76.9%, respectively ( $P = 0.015$ ), and the prevalence of iron deficiency anemia (hemoglobin < 110 g/L and ferritin < 12  $\mu$ g/L) was 44.3 and 45.4%, respectively ( $P = 0.92$ ). The relatively higher prevalence of anemia among HIV-infected infants was attributed to the anemia of chronic disease. Among infants with and without iron deficiency, the fitted regression line was  $\log_{10}$  plasma erythropoietin =  $2.86 - 0.016 \cdot \text{hemoglobin}$ , and  $\log_{10}$  plasma erythropoietin =  $4.11 - 0.028 \cdot \text{hemoglobin}$ , respectively, with a difference in the slope of the regression lines between  $\log_{10}$  erythropoietin and hemoglobin among infants with and without iron deficiency ( $P = 0.049$ ). Infants in Uganda have an extremely high prevalence of anemia, and nearly half of the anemia is due to iron deficiency. The erythropoietin response to anemia appears to be upregulated among infants with iron deficiency. *J. Nutr.* 132: 423–429, 2002.

**KEY WORDS:** • anemia • erythropoietin • hemoglobin • HIV • iron

Anemia is common during human immunodeficiency virus (HIV)<sup>3</sup> infection in both children and adults. Several studies have shown that anemia is associated with decreased survival during HIV infection (1), and recently, increased mortality was linked with anemia in a large multicenter cohort of women (2). A higher prevalence of anemia has been described among HIV-infected children who died early compared with long-term survivors (3). Anemia has not been well characterized among HIV-infected infants, especially in sub-Saharan Africa where the majority of infected infants are found. The pathogenesis of anemia during HIV infection is often multifactorial, and contributing factors include iron deficiency, the anemia of chronic disease associated with HIV, malaria and opportunistic infections (4). The relative contribution of iron deficiency to the anemia of HIV-infected infants in developing countries is not well understood.

The anemia of chronic disease is characterized by an increase in circulating inflammatory cytokines, immune activation and in some cases, by a blunted response of erythropoietin to anemia (5). Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), an inflammatory cytokine, may play a role in the suppression of erythropoiesis during HIV infection (6–8). A negative correlation was described between hemoglobin and circulating receptors for TNF- $\alpha$  in adults with advanced HIV disease, suggesting a possible role of TNF- $\alpha$  in the impairment of erythropoietin production and erythropoiesis (9). Neopterin, a marker of macrophage activation, has been found to be associated with suppression of erythropoietin among HIV-infected children with hemophilia (10). A blunted response to erythropoietin has been described among HIV-infected adults (11,12). Serum erythropoietin concentrations among anemic, HIV-infected children in Canada and the Bahamas were lower than those reported for children with various types of anemia, including iron deficiency anemia (13), but a recent study did not show evidence for a blunted response to erythropoietin among HIV-infected infants in Malawi (14).

The relationships among plasma erythropoietin concentrations, TNF- $\alpha$ , neopterin, HIV load, iron deficiency and anemia in HIV-infected infants are not well understood. The specific aim of this study was to characterize the relative contribution of iron deficiency and chronic disease to the

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<sup>3</sup> Abbreviations used: AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus; PCR, polymerase chain reaction; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .

anemia in HIV-infected and uninfected infants in Kampala, Uganda.

## SUBJECTS AND METHODS

The study population consisted of 9-mo-old, HIV-infected infants ( $n = 165$ ) and uninfected infants ( $n = 39$ ) born to HIV-infected mothers and seen at Mulago Hospital, in Kampala, Uganda. Between January 1995 and June 1998, after written informed consent was obtained, women presenting at Mulago Hospital for antenatal care were offered pre- and post-test HIV counseling and acquired immunodeficiency syndrome (AIDS) education. Women were considered HIV-positive on the basis of positive results on two different ELISA for HIV-1 antibody (Vironostika, Organon-Teknika, Durham, NC; Murex HIV1 + 2, Murex Diagnostics, Dartford, UK). Samples were considered negative if the first test, HIV-1 Vironostika ELISA, was negative, or indeterminate if other results were obtained. After written, informed consent from the mother, a venous blood sample was obtained from the infant. Infants were screened at 3–5 mo of age for HIV-1 infection using qualitative HIV-1 RNA polymerase chain reaction (PCR) followed by a quantitative HIV-1 RNA PCR (Roche Amplicor Monitor, Roche Diagnostics, Indianapolis, IN). Infant length and weight were measured using standard anthropometric techniques (15). No infants were receiving antiretroviral medications during the study period.

At 9 mo of age, hemoglobin concentrations were measured by an automated T540 hematology analyzer (Coulter Diagnostics, Hialeah, FL). Plasma was immediately divided into aliquots and stored at  $-70^{\circ}\text{C}$  until subsequent laboratory analyses. Plasma erythropoietin (ALPCO, Windham, NH), TNF- $\alpha$  (Human TNF- $\alpha$ , Quantikine High Sensitivity, R & D Systems, Minneapolis, MN), neopterin (ALPCO) and ferritin (Human Ferritin Enzyme Immunoassay Test Kit, ALPCO) concentrations were measured by ELISA. Pooled human standards were used to measure intra- and interassay CV in laboratory analyses. The intra-assay and interassay CV for erythropoietin, TNF- $\alpha$ , neopterin, and ferritin were 6.6 and 8.3, 6.2 and 8.1, 1.6 and 5.0, and 5.6 and 7.2, respectively. Plasma erythropoietin, TNF- $\alpha$ , neopterin, ferritin and HIV load could not be measured on all infants due to the limited amount of plasma drawn from some of the infants. Plasma HIV load at 9 mo was measured using quantitative HIV-1 RNA PCR (Roche Amplicor Monitor, version 1.5) with a sensitivity limit of  $\sim 400$  HIV RNA copies/mL. The study protocol was approved by the Joint Committee on Clinical Investigation at Johns Hopkins University and the Uganda National AIDS Subcommittee with final approval by the Office for Protection from Research Risk, National Institutes of Health, Bethesda, MD.

Growth standards from the National Center for Health Statistics were used as reference (17). Weight-for-age Z-score less than  $-2$  SD, weight-for-length Z-score less than  $-2$  SD and length-for-age Z-score less than  $-2$  SD were considered consistent with underweight, wasting and stunting, respectively, as per convention (16). Anemia was defined as hemoglobin  $< 110$  g/L (17), and moderate-to-severe anemia was defined as hemoglobin  $< 90$  g/L as per convention. Hematology reference ranges for normal, healthy 6- to 12-mo-old infants were used for defining RBC indices (18). Microcytic was defined as mean cell volume  $< 70$  fL and hypochromic as mean cell hemoglobin concentration  $< 32.4$  g/dL (18). Iron deficiency was defined as plasma ferritin  $< 12$   $\mu\text{g/L}$ , and iron deficiency anemia was defined as iron deficiency with anemia (hemoglobin  $< 110$  g/L) (19). The sample size of the study was based upon 90% power to detect a 5 g/L difference in hemoglobin between HIV-positive and HIV-negative infants, with  $\alpha = 0.05$  and a two-sided test, assuming normal distribution and equal variances. Comparisons between continuous variables were made using Student's  $t$  test. Appropriate variable transformations were made for skewed data, such as  $\log_{10}$  transformation for plasma HIV load, ferritin, erythropoietin, TNF- $\alpha$  and neopterin. Comparisons of categorical data were made using  $\chi$ -square or exact tests. Spearman correlation was used to examine the correlation between selected variables.

A linear regression model was used to compare the relationship between plasma erythropoietin and hemoglobin concentrations among infants with and without iron deficiency using the model  $\log_{10}$

erythropoietin =  $\beta_0 + \beta_1$ hemoglobin +  $\beta_2$ iron deficiency +  $\beta_3$ iron deficiency  $\cdot$  hemoglobin +  $\epsilon$ , where iron deficiency = 0 or 1 and hemoglobin was expressed in g/L. The statistical software package SAS (version 8.1, SAS Institute Cary, NC) and STATA (College Station, TX) were used for all analyses.

## RESULTS

The characteristics of HIV-positive and HIV-negative 9-mo-old infants are shown in **Table 1**. There were no differences between groups in age, sex distribution or birth weight. The prevalence of underweight and wasting was higher among HIV-positive than HIV-negative infants. Mean hemoglobin concentrations were lower and the prevalence of anemia was higher among HIV-positive than HIV-negative infants. Mean cell volume and mean cell hemoglobin were not significantly different between the two groups, but mean cell hemoglobin concentration was significantly lower among HIV-positive compared with HIV-negative infants. The prevalence of iron deficiency and iron deficiency anemia was not different between HIV-positive and HIV-negative infants.  $\log_{10}$  neopterin concentrations were higher among HIV-positive compared with HIV-negative infants, and these data were suggestive that  $\log_{10}$  TNF- $\alpha$  concentrations were relatively higher among HIV-positive infants ( $P = 0.08$ ).

In children with HIV infection, the relationships among anemia, iron status, markers of immune activation and inflammation, anthropometry, and two indicators of HIV disease severity, CD4<sup>+</sup> lymphocyte count and plasma HIV load, are shown in **Table 2**. Comparisons were made between infants with CD4<sup>+</sup> lymphocyte count above and below the median of 1404 cells/ $\mu\text{L}$  and between infants with plasma HIV load above and below the median of 1,119,000 copies/mL. Infants with CD4<sup>+</sup> lymphocyte count below the median of 1404 cells/ $\mu\text{L}$  and infants with plasma HIV load above the median of 1,119,000 copies/mL were defined as having more severe HIV disease. In general, the prevalence of underweight, wasting and stunting was higher among infants with more severe HIV disease. Mean hemoglobin and the prevalence of anemia were not significantly different by HIV disease severity.  $\log_{10}$  ferritin concentrations were significantly higher among infants below compared with infants above the median CD4<sup>+</sup> lymphocyte count, but there were no differences in  $\log_{10}$  ferritin concentrations by category of plasma HIV load. The prevalence of infants with iron deficiency and iron deficiency anemia was significantly lower among infants below compared with infants above the median CD4<sup>+</sup> lymphocyte count.  $\log_{10}$  neopterin was significantly higher among infants with more severe HIV disease. There were no differences in TNF- $\alpha$  concentrations by CD4<sup>+</sup> lymphocyte count category, but TNF- $\alpha$  concentrations were significantly higher among infants in the category with higher plasma HIV load.

Birth weight, anthropometric measurements, RBC indices, iron status, markers of immune activation and inflammation, CD4<sup>+</sup> lymphocyte count and plasma HIV load were compared between HIV-infected children with and without moderate-to-severe anemia (**Table 3**). HIV-infected infants with moderate-to-severe anemia had significantly lower birth weight, and these data were suggestive that both weight-for-age Z-score ( $P = 0.054$ ) and length-for-age Z-score ( $P = 0.053$ ) were also lower among infants with than without moderate-to-severe anemia. Microcytic, hypochromic anemia was more prevalent among infants with moderate-to-severe anemia. The prevalence of iron deficiency, mean  $\log_{10}$  TNF- $\alpha$  concentrations, CD4<sup>+</sup> lymphocyte counts and  $\log_{10}$  HIV load was not different between HIV-infected infants with and without mod-

TABLE 1

Characteristics of 9-mo-old infants with and without human immunodeficiency virus (HIV) infection<sup>1</sup>

Characteristic	HIV-positive	HIV-negative	P-value
Age, d	278 ± 12 (165)	287 ± 11 (39)	0.77
Sex, % female	52.7 (165)	56.4 (39)	0.67
Birth weight, g	3112 ± 542 (151)	3165 ± 684 (34)	0.62
Weight-for-age Z-score	-1.73 ± 1.34 (165)	-1.13 ± 1.21 (39)	0.01
Weight-for-length Z-score	-0.47 ± 1.18 (165)	0.29 ± 0.96 (39)	0.0005
Length-for-age Z-score	-1.74 ± 1.3 (165)	-1.64 ± 1.37 (39)	0.76
Weight-for-age Z-score < -2 SD, %	42.4 (165)	25.6 (39)	0.054
Weight-for-length Z-score < -2 SD, %	12.7 (165)	0 (39)	0.02
Length-for-age Z-score < -2 SD, %	39.4 (165)	33.3 (39)	0.48
Hemoglobin, g/L	94 ± 12 (165)	101 ± 13 (39)	0.002
Hemoglobin < 110 g/L, %	90.9 (165)	76.9 (39)	0.015
Hemoglobin < 90 g/L, %	35.1 (165)	20.5 (39)	0.08
MCV, <sup>2</sup> fL	68.6 ± 7.4 (165)	68.7 ± 7.4 (39)	0.92
MCV <sup>3</sup> < 70 fL, %	60.6 (165)	58.9 (39)	0.85
MCHC, g/dL	31.3 ± 1.6 (165)	32.4 ± 2.1 (39)	0.006
MCHC < 32.4 g/dL, %	77.5 (165)	38.4 (39)	0.001
MCH, pg	21.5 ± 2.9 (165)	22.2 ± 2.7 (39)	0.2
MCH < 25 pg, %	86.0 (165)	82.0 (39)	0.52
Microcytic, hypochromic anemia, <sup>4</sup> %	80.0 (165)	53.8 (39)	0.001
Log <sub>10</sub> ferritin, µg/L	1.15 ± 0.58 (140)	0.92 ± 0.41 (22)	0.08
Iron deficiency, %	47.1 (140)	59.1 (22)	0.29
Iron deficiency anemia, %	44.3 (140)	45.4 (22)	0.92
Log <sub>10</sub> erythropoietin, mU/mL	1.35 ± 0.67 (140)	1.33 ± 0.44 (22)	0.76
Log <sub>10</sub> TNF-α, pg/mL	1.34 ± 0.22 (140)	1.25 ± 0.23 (22)	0.08
Log <sub>10</sub> neopterin, nmol/L	1.45 ± 0.23 (140)	1.23 ± 0.26 (22)	0.0001
CD4 <sup>+</sup> lymphocyte count, cells/µL	1489 ± 822 (165)	2765 ± 2503 (39)	0.003
Log <sub>10</sub> HIV load, copies/mL	5.93 ± 0.61 (144)	—	—

<sup>1</sup> Values are means ± SD (n) or % (n).

<sup>2</sup> Abbreviations: TNF-α, tumor necrosis factor-α; MCV, mean cell volume; MCHC, mean cell hemoglobin concentration; MCH, mean cell hemoglobin.

<sup>3</sup> Based on reference values in Ref. 19.

<sup>4</sup> Defined as hemoglobin < 110 g/L, MCV < 70 fL and MCHC < 32.5 g/dL.

erate-to-severe anemia. Log<sub>10</sub> neopterin concentrations were significantly higher among infants with than without moderate-to-severe anemia.

Spearman correlations between hemoglobin, erythropoietin, HIV load, CD4<sup>+</sup> lymphocyte count, TNF-α, neopterin and ferritin among HIV-infected infants are shown in **Table 4**. Hemoglobin had a significant negative correlation ( $r = -0.592$ ,  $P < 0.0001$ ) with plasma erythropoietin concentrations. Plasma HIV load was significantly correlated with neopterin ( $r = 0.300$ ,  $P < 0.001$ ) and TNF-α ( $r = 0.342$ ,  $P < 0.0001$ ). There was a significant positive correlation between neopterin and TNF-α ( $r = 0.264$ ,  $P < 0.002$ ). Ferritin had a significant negative correlation with CD4<sup>+</sup> lymphocyte count ( $r = -0.277$ ,  $P < 0.0009$ ) and with neopterin ( $r = 0.408$ ,  $P < 0.0001$ ).

The relationship between log<sub>10</sub> plasma erythropoietin and hemoglobin concentrations among HIV-infected infants with and without iron deficiency is shown in **Figure 1**. The fitted regression lines were log<sub>10</sub> plasma erythropoietin = 4.11 - 0.028 · hemoglobin for HIV-infected infants with iron deficiency and log<sub>10</sub> plasma erythropoietin = 2.86 - 0.016 · hemoglobin for HIV-infected infants without iron deficiency. The slopes of the regression lines between log<sub>10</sub> erythropoietin and hemoglobin among infants with and without iron deficiency were different from each other by multiple linear regression ( $P = 0.049$ ).

## DISCUSSION

To our knowledge, this is the first study to characterize iron status, erythropoietin, immunological factors and anemia

among HIV-infected infants in sub-Saharan Africa. The prevalence of anemia of >90% among HIV-infected infants in Uganda was much higher than has been reported among similar populations of HIV-infected infants elsewhere. The study in Uganda consisted of infants who were born to HIV-infected mothers, followed from birth and not selected on the basis of symptoms. In a study of HIV-infected infants in New Haven, CT who were followed since early infancy and also not selected on the basis of symptoms, 32.9% were found to be anemic at 9 mo of age (20). Studies of symptomatic HIV-infected infants have shown a relatively higher prevalence of anemia. In a clinic-based study in New York City, anemia was reported in 100 and 92% of symptomatic HIV-infected children with and without opportunistic infections, respectively (21). Anemia was found in 84% of HIV-infected children in Zimbabwe who were hospitalized (22). A study from Abidjan, Ivory Coast, suggested that the clinical diagnosis of anemia was the main admitting diagnosis for hospitalization for 12.2% of HIV-positive children (23). Anemia was the third main admission diagnosis for HIV-infected children, after acute respiratory infection and malnutrition (23).

Iron deficiency anemia contributed to at least half of the anemia among these 9-mo-old infants in Uganda. The proportions of infants with iron deficiency and iron deficiency anemia were not different between HIV-positive and HIV-negative infants. Ferritin is a positive acute phase reactant (24); thus, among infants with more severe HIV infection, use of ferritin as an indicator of iron status is more limited and may underestimate the proportion of infants with iron deficiency and iron deficiency anemia. There was a significant, positive

TABLE 2

Relationship between anemia and indicators of human immunodeficiency virus (HIV) disease progression among infants<sup>1</sup>

Characteristic	CD4 lymphocyte count (cells/ $\mu$ L) <sup>2</sup>		P value	Plasma HIV load (copies/mL) <sup>2</sup>		P-value
	<1404	$\geq$ 1404		$\geq$ 1,119,000	<1,119,000	
Age, d	278 $\pm$ 12 (82)	278 $\pm$ 12 (83)	0.98	279 $\pm$ 12 (72)	279 $\pm$ 13 (72)	0.88
Sex, % female	42.5 (82)	57.4 (83)	0.04	48.6 (72)	61.1 (72)	0.13
Weight-for-age Z-score	-2.19 $\pm$ 1.35 (82)	-1.28 $\pm$ 1.17 (83)	0.0001	-2.11 $\pm$ 1.25 (72)	-1.39 $\pm$ 1.35 (72)	0.002
Weight-for-length Z-score	-0.70 $\pm$ 1.29 (82)	-0.24 $\pm$ 1.02 (83)	0.02	-0.62 $\pm$ 1.22 (72)	-0.33 $\pm$ 1.17 (72)	0.15
Length-for-age Z-score	-2.09 $\pm$ 1.36 (82)	-1.38 $\pm$ 1.13 (83)	0.0004	-2.08 $\pm$ 1.29 (72)	-1.41 $\pm$ 1.29 (72)	0.002
Weight-for-age Z-score < -2 SD, %	54.8 (82)	30.1 (83)	0.001	51.4 (72)	33.3 (72)	0.028
Weight-for-length Z-score < -2 SD, %	20.7 (82)	4.8 (83)	0.001	12.5 (72)	12.5 (72)	1.00
Length-for-age Z-score < -2 SD, %	51.2 (82)	27.7 (83)	0.002	54.1 (72)	27.8 (72)	0.001
Mean hemoglobin, g/L	94 $\pm$ 12 (82)	93 $\pm$ 13 (83)	0.74	94 $\pm$ 13 (72)	94 $\pm$ 13 (72)	0.78
Hemoglobin < 110 g/L, %	87.8 (82)	93.9 (83)	0.17	90.2 (72)	90.2 (72)	1.00
Hemoglobin < 90 g/L, %	37.8 (82)	32.5 (83)	0.47	37.5 (72)	34.7 (72)	0.73
MCV, <sup>3</sup> fL	69.6 $\pm$ 7.0 (82)	67.5 $\pm$ 7.7 (83)	0.063	69.3 $\pm$ 7.8 (72)	68.0 $\pm$ 7.1 (72)	0.29
MCV < 70 fL, <sup>4</sup> %	54.8 (82)	66.2 (83)	0.13	61.1 (72)	62.5 (72)	0.86
MCHC, g/dL	31.5 $\pm$ 1.5 (82)	31.1 $\pm$ 1.6 (83)	0.14	31.3 $\pm$ 1.6 (72)	31.1 $\pm$ 1.5 (72)	0.43
MCHC < 32.4 g/dL, %	79.2 (82)	75.9 (83)	0.60	73.6 (72)	83.3 (72)	0.15
MCH, pg	22.0 $\pm$ 2.7 (82)	21.1 $\pm$ 3.0 (83)	0.043	21.7 $\pm$ 3.1 (72)	21.2 $\pm$ 2.7 (72)	0.24
MCH < 25 pg, %	85.3 (82)	86.7 (83)	0.79	83.3 (72)	88.9 (72)	0.33
Microcytic, hypochromic anemia, <sup>5</sup> %	78.0 (82)	81.9 (83)	0.53	76.4 (72)	83.3 (72)	0.29
Log <sub>10</sub> ferritin, $\mu$ g/L	1.28 $\pm$ 0.56 (70)	1.01 $\pm$ 0.56 (70)	0.006	1.22 $\pm$ 0.55 (69)	1.07 $\pm$ 0.60 (70)	0.11
Iron deficiency, %	35.7 (70)	58.5 (70)	0.007	43.5 (69)	51.4 (70)	0.34
Iron deficiency anemia, %	30.0 (70)	58.7 (70)	0.001	42.0 (69)	47.1 (70)	0.54
Log <sub>10</sub> erythropoietin, mU/mL	1.29 $\pm$ 0.73 (73)	1.43 $\pm$ 0.60 (72)	0.21	1.38 $\pm$ 0.60 (72)	1.33 $\pm$ 0.73 (72)	0.64
Log <sub>10</sub> TNF- $\alpha$ , pg/mL	1.36 $\pm$ 0.22 (73)	1.32 $\pm$ 0.22 (72)	0.30	1.41 $\pm$ 0.21 (72)	1.27 $\pm$ 0.21 (72)	0.0003
Log <sub>10</sub> neopterin, nmol/L	1.51 $\pm$ 0.25 (73)	1.39 $\pm$ 0.19 (72)	0.002	1.52 $\pm$ 0.23 (72)	1.39 $\pm$ 0.22 (72)	0.002

<sup>1</sup> Values are means  $\pm$  SD (n) or % (n).

<sup>2</sup> Groups divided above and below the median.

<sup>3</sup> Abbreviations: TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; MCV, mean cell volume; MCHC, mean cell hemoglobin concentration; MCH, mean cell hemoglobin.

<sup>4</sup> Based upon reference values in Ref. 19.

<sup>5</sup> Defined as hemoglobin < 110 g/L, MCV < 70 fL and MCHC < 32.5 g/dL.

correlation of ferritin with neopterin, an indicator of immune activation. Higher ferritin concentrations were also associated with lower CD4<sup>+</sup> lymphocyte count, suggesting that more severe HIV infection and inflammation are associated with an elevation in ferritin. Thus, the prevalence of iron deficiency and iron deficiency anemia may have been underestimated among infants with lower CD4<sup>+</sup> lymphocyte counts. This study is limited in that acute phase proteins were not measured; this might have provided more insight into the prevalence of iron deficiency among those without elevated acute phase proteins.

Plasma HIV load was significantly correlated with plasma neopterin, a marker of macrophage activation, and with TNF- $\alpha$ , an inflammatory cytokine that has been implicated in the suppression of erythropoiesis. Among infants who have perinatally acquired HIV infection, plasma HIV load concentrations are known to reach a high peak and then decline slowly, and infants with a better prognosis have a steeper decline in HIV load (25). Two markers for advanced HIV disease in infants, high plasma HIV load and low CD4<sup>+</sup> lymphocyte count (25–27), were not significantly related to hemoglobin concentrations. These results were surprising because it was expected that the degree of anemia would be worse in infants with a higher HIV load. Although elevated plasma TNF- $\alpha$  has been implicated in the suppression of erythropoiesis (4,7), the present study suggests that immune activation, as indicated by elevated neopterin concentrations, has a significant association with moderate-to-severe anemia. The anemia of chronic disease probably accounts for the higher prevalence of anemia among HIV-positive infants com-

pared with HIV-negative infants. The prevalence of low birth weight, underweight and stunting seemed to be relatively higher among HIV-positive infants who had moderate-to-severe anemia, and these data are also suggestive that the burden of chronic disease may be higher among infants who are more anemic.

A limitation of this study is that blood smears for malaria parasitemia were not obtained from infants at 9 mo of age; thus, the relationship between malaria infection and anemia could not be assessed directly. These infants came from a study population in which *Plasmodium falciparum* malaria is endemic. In a previous study conducted in the same clinic and study population, 458 children with and without HIV infection were followed from early infancy through 4 y, and 30% of infants had at least one episode of smear-confirmed malaria during the first 12 mo of life (28).

To our knowledge, this is the first study to compare the erythropoietin response to anemia among infants with and without iron deficiency in sub-Saharan Africa. Erythropoietin production by the kidney is influenced by hemoglobin concentrations, and it is considered more appropriate in the comparison of two groups to compare the slope of the regression line between log<sub>10</sub> erythropoietin and hemoglobin rather than the absolute concentrations of erythropoietin (29). In a study of 73 HIV-positive and 246 HIV-negative 12-mo-old infants in Malawi, no differences were found in the slopes of the regression lines between log<sub>10</sub> erythropoietin and hemoglobin (-0.011 and -0.013, respectively), suggesting that the erythropoietin response to anemia is not blunted among HIV-positive infants (14). In contrast, a blunted erythropoietin

TABLE 3

Indicators of iron status, inflammation and immune activation in moderate to severely anemic human immunodeficiency virus (HIV)-infected infants<sup>1</sup>

Characteristic	Hemoglobin < 90 g/L	Hemoglobin ≥ 90 g/L	P-value
Age, d	280 ± 13 (58)	278 ± 11 (107)	0.34
Sex, % female	46.5 (58)	56.1 (107)	0.24
Birth weight, g	2978 ± 463 (52)	3182 ± 569 (99)	0.027
Weight-for-age Z-score	-2.02 ± 1.41 (58)	-1.58 ± 1.29 (107)	0.054
Weight-for-length Z-score	-0.70 ± 1.07 (58)	-0.34 ± 1.22 (107)	0.053
Length-for-age Z-score	-1.88 ± 1.43 (58)	-1.66 ± 1.22 (107)	0.31
Weight-for-age Z-score < -2 SD, %	50.0 (58)	38.3 (107)	0.14
Weight-for-length Z-score < -2 SD, %	17.2 (58)	10.2 (107)	0.20
Length-for-age Z-score < -2 SD, %	46.5 (58)	35.3 (107)	0.16
MCV, <sup>2</sup> fL	65.3 ± 8.0 (58)	70.4 ± 6.4 (107)	0.0001
MCV < 70 fL, <sup>3</sup> %	74.1 (58)	53.2 (107)	0.009
MCHC, g/dL	30.6 ± 1.59 (58)	31.7 ± 1.51 (107)	0.0001
MCHC < 32.4 g/dL, %	91.3 (58)	70.1 (107)	0.002
MCH, pg	20.0 ± 2.9 (58)	22.3 ± 2.5 (107)	0.0001
MCH < 25 pg, %	94.8 (58)	81.3 (107)	0.017
Microcytic, hypochromic anemia, <sup>4</sup> %	94.8 (58)	71.9 (107)	0.001
Log <sub>10</sub> ferritin, μg/L	1.04 ± 0.69 (50)	1.21 ± 0.50 (90)	0.10
Iron deficiency, %	54.0 (50)	43.3 (90)	0.22
Log <sub>10</sub> erythropoietin, mU/mL	1.77 ± 0.50 (53)	1.12 ± 0.64 (92)	0.0001
Log <sub>10</sub> TNF-α, pg/mL	1.32 ± 0.21 (53)	1.35 ± 0.23 (92)	0.49
Log <sub>10</sub> neopterin, nmol/L	1.51 ± 0.23 (53)	1.42 ± 0.23 (92)	0.04
CD4 <sup>+</sup> lymphocyte count, cells/μL	1343 ± 758 (58)	1569 ± 848 (107)	0.08
Log <sub>10</sub> HIV load, copies/mL	5.99 ± 0.46 (52)	5.90 ± 0.67 (92)	0.34

<sup>1</sup> Values are means ± SD (n) or % (n).

<sup>2</sup> Abbreviations: TNF-α, tumor necrosis factor-α; MCV, mean cell volume; MCHC, mean cell hemoglobin concentration; MCH, mean cell hemoglobin.

<sup>3</sup> Based upon reference values in Ref. 19.

<sup>4</sup> Defined as hemoglobin < 110 g/L, MCV < 70 fL and MCHC < 32.5 g/dL.

response to anemia has been described in HIV-infected adults (11). A direct comparison of the slopes of the regression lines between log<sub>10</sub> erythropoietin and hemoglobin between some studies is limited by the use of different assays for the measurement of erythropoietin (11).

We examined the erythropoietin response to anemia among infants with and without iron deficiency, because hypoxia-inducible factor-1 is involved in transcriptional activation of the erythropoietin gene (30) and is influenced by iron status (31). In the present study, the slope of the regression line between log<sub>10</sub> erythropoietin and hemoglobin was signif-

icantly more negative among infants with iron deficiency compared with infants without iron deficiency, suggesting that iron deficiency may possibly upregulate the erythropoietin response of the kidneys to low hemoglobin concentrations.

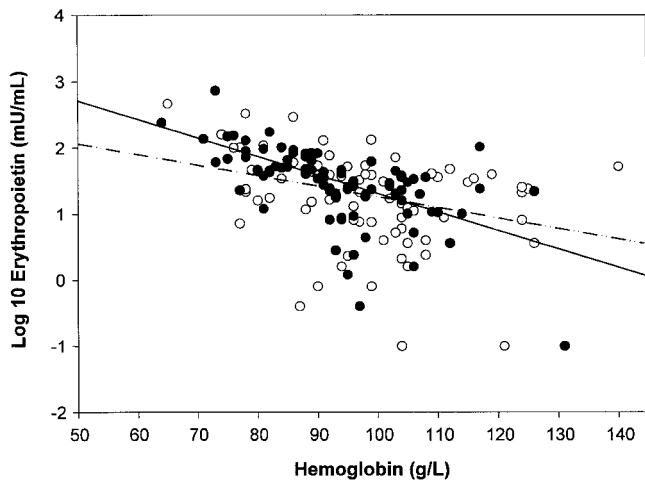
Although iron supplementation is often used to treat anemia in HIV-infected infants, it is unclear how much the anemia responds to iron supplementation among these infants and whether other laboratory indicators of iron status behave as they would among uninfected infants. A study from Italy suggests that intestinal malabsorption of iron is common among anemic HIV-infected infants, and that iron supplemen-

TABLE 4

Spearman correlations for hemoglobin and other variables among human immunodeficiency virus (HIV)-positive 9-mo-old infants

	CD4 count	Hemoglobin	Erythropoietin	Neopterin	TNF-α <sup>1</sup>	Ferritin
	<i>r(n)</i>					
HIV load	-0.202 (145) P = 0.02	0.001 (144) P = 0.98	0.021 (144) P = 0.79	0.300 (144) P < 0.001	0.342 (144) P < 0.0001	0.141 (139) P = 0.09
CD4 count		0.042 (165) P = 0.58	0.075 (145) P = 0.36	-0.234 (145) P = 0.005	-0.143 (144) P = 0.08	-0.277 (140) P < 0.0009
Hemoglobin			-0.592 (145) P < 0.0001	-0.110 (145) P = 0.18	0.085 (145) P = 0.26	0.131 (140) P = 0.12
Erythropoietin				0.135 (145) P = 0.10	0.044 (145) P = 0.59	-0.196 (140) P = 0.02
Neopterin					0.264 (145) P = 0.002	0.408 (140) P < 0.0001
TNF-α						0.102 (140) P = 0.22

<sup>1</sup> TNF-α, tumor necrosis factor-α.



**FIGURE 1** Relationship between  $\log_{10}$  plasma erythropoietin and hemoglobin among infants with (filled circles, solid line) and without (open circles, broken line) iron deficiency. The fitted regression lines were  $\log_{10}$  plasma erythropoietin =  $4.11 - 0.028 \cdot \text{hemoglobin}$  and  $\log_{10}$  plasma erythropoietin =  $2.86 - 0.016 \cdot \text{hemoglobin}$ , respectively, with a difference in the slope of the regression lines between  $\log_{10}$  erythropoietin and hemoglobin among infants with and without iron deficiency ( $P = 0.049$ ).

tation increases hemoglobin in about half of infants with iron deficiency anemia (32). Other micronutrient deficiencies such as vitamin A deficiency (33) may also influence iron metabolism. Recently, concern has been raised about iron supplementation during HIV infection because some epidemiologic studies suggest that iron supplementation and overload may worsen the course of HIV infection (34,35). No relationship was found between iron status and HIV disease severity among HIV-positive women in sub-Saharan Africa (36), and there is little evidence to contraindicate the use of iron supplementation for iron deficiency among HIV-positive infants in developing countries (37). Anemia in infants and young children is associated with retarded psychomotor development and impaired cognitive behavior (38), and several studies have shown an association between anemia and mortality during HIV infection (1,2,4). The potential risks and benefits of iron supplementation for HIV-infected infants and children may require further evaluation through controlled clinical trials.

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